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Promotion of Lung Tumors in Mice

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Several elements of two-stage carcinogenesis apply to the development of lung tumors in Strain A or Swiss-Webster mice. At least three agents which have been identified as promoters in skin, urinary bladder and liver will also enhance tumor formation in lung: phorbol, saccharin and butylated hydroxytoluene (BHT). The antioxidant BHT acts in many respects like a typical promoting agent: it is effective if animals are treated after exposure to an initiating agent, but not if they are treated beforehand. Administration of BHT can be delayed up to 5 months after urethan treatment and still enhance tumor formation. BHT enhances lung tumor formation regardless of its route of administration (IP injection, gavage, or ingestion in the diet). The lowest dose of BHT required to produce an effect has not yet been determined. In at least one mouse strain, BHT also enhances tumor formation in animals initiated with 3-methylcholanthrene or dimethylnitrosamine.

On the other hand, no evidence is available yet to show that BHT would enhance tumor development in animals treated with subcarcinogenic doses of an initiating compound. Nor has it been possible to produce more tumors with BHT in mouse strains which have a low spontaneous tumor incidence and respond poorly to urethan. The question has not been resolved whether BHT accelerates growth of preformed tumors only or whether it induces the formation of more tumors. Nevertheless, the data collected on the effects of BHT on mouse lung tumor development have broadened the concept of two-stage carcinogenesis and complement the evidence for initiation- promotion available for other epithelial tissues such as liver, colon, stomach, trachea, urinary bladder and mammary gland.

Introduction

In 1972, Armuth and Berenblum (*1*) provided the first evidence that the development of lung tumors in mice might be enhanced by administration of a promoting agent. When dimethylnitrosamine (DMN) was injected into newborn AKR mice and the mice treated subsequently with phorbol, both tumor incidence and tumor multiplicity increased. When DMN exposure was delayed until 10 days after birth, no promoting effect of phorbol could be observed. The authors concluded that phorbol, the well known promoter of skin tumors in mice, might also act as a systemic promoting agent in mouse lung provided the initiating stimulus was given early after birth (*1*). In 1977 it was reported that the development of lung tumors in Strain A and Swiss- Webster mice could be enhanced by repeated injections of the hindered phenolic antioxidant butylated hydroxytoluene (BHT) (*2*). A third chemical suspected to be a promoting agent, saccharin (*3*), was found to influence tumor development in lung. Strain A mice were pretreated for 1 week with four different commercial preparations of the artificial sweetener, given one single injection of urethan (ethyl carbamate) and treated for another 16 weeks with saccharin. When the animals were killed it was found that the animals treated with both urethan and saccharin had two to four times as many tumors per lung as had animals treated with urethan alone (*4*).

There is thus evidence available to show that two stage carcinogenesis may occur in mouse lung. Several studies with BHT have now been done and the data are discussed in some detail. The available information seems to suggest that the mouse lung-BHT system can be taken as a valid example of two-stage carcinogenesis.

Tumor Enhancement in Mouse Lung

Temporal Relationship

The following experiments show that the development of lung tumors in mice can be enhanced by systemic administration of BHT. If certain mouse strains, such as Strain A or Swiss Webster mice, are treated with a variety of carcinogens, numerous lung tumors develop in virtually all animals within 4 to 6 months (*5*). The number of tumors is proportional to the amount of carcinogen administered.

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One of the most effective agents is urethan. If Swiss Webster mice or Strain A mice are injected with a carcinogenic dose of urethan and subsequently treated repeatedly with BHT, significantly more tumors are found 4-6 months later compared to appropriate controls. This was found to be true whether BHT was injected intraperitoneally (2), given by stomach tube (6) or offered to the animals in the diet (7). Figure 1 shows the results of three such experiments. In none of these experiments was it found that BHT would influence tumor incidence or tumor multiplicity if mice were treated with NaCl instead of with urethan. This is in agreement with other studies, where it was found that BHT failed to produce lung tumors in mice (8).

On the other hand, pretreatment with BHT does not influence tumor development. Swiss Webster mice were given 13 weekly injections of BHT. One week after the last BHT injection urethan was administered, and the tumors per lung were counted 4 months later. No difference was found between animals exposed to BHT and given urethan and animals pretreated with corn oil before urethan injection (9). In a second experiment, animals were fed a diet containing 0.75% BHT for 2 weeks and then injected with a single dose of urethan. Again, no difference was found in animals pre-exposed to BHT compared to the appropriate control groups (7).

Several experiments have now shown that BHT effectively enhances tumor formation in mouse lung provided the antioxidant treatment is begun after administration of urethan. If the temporal sequence of exposure is reversed, BHT has no effect. The system meets therefore one important criterion usually attributed to promoting agents: BHT is effective only if given after initiation of tumor formation in mouse lung by a carcinogen, but not if given prior to the carcinogen. BHT alone is not carcinogenic.

A second question is whether delay of BHT exposure up to several weeks after urethan still might influence tumor development. First it was shown that beginning BHT administration 6 weeks after urethan still enhanced tumor formation (9). In a more recent study, mice were given 1000 mg/kg of urethan as an initiating dose (10). BHT treatments (300 mg/kg IP, once a week for a total of four

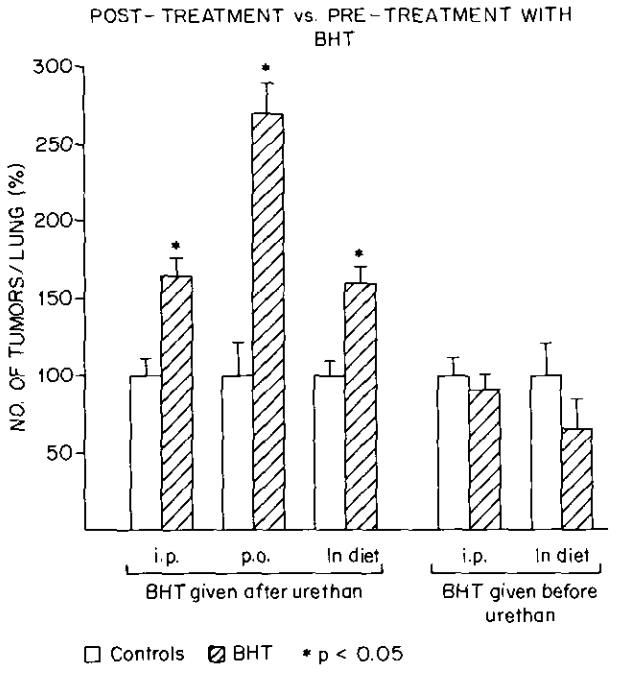

FIGURE 1. Swiss-Webster or Strain A mice were given BHT before or after a carcinogenic dose of urethan: (▨) with BHT; (□) control. Posttreatment with BHT enhanced tumor development significantly, whereas pretreatment had no effect. Asterisks (*) denote $p<0.05$. Data from Witschi et al. (2, 6, 7, 9).

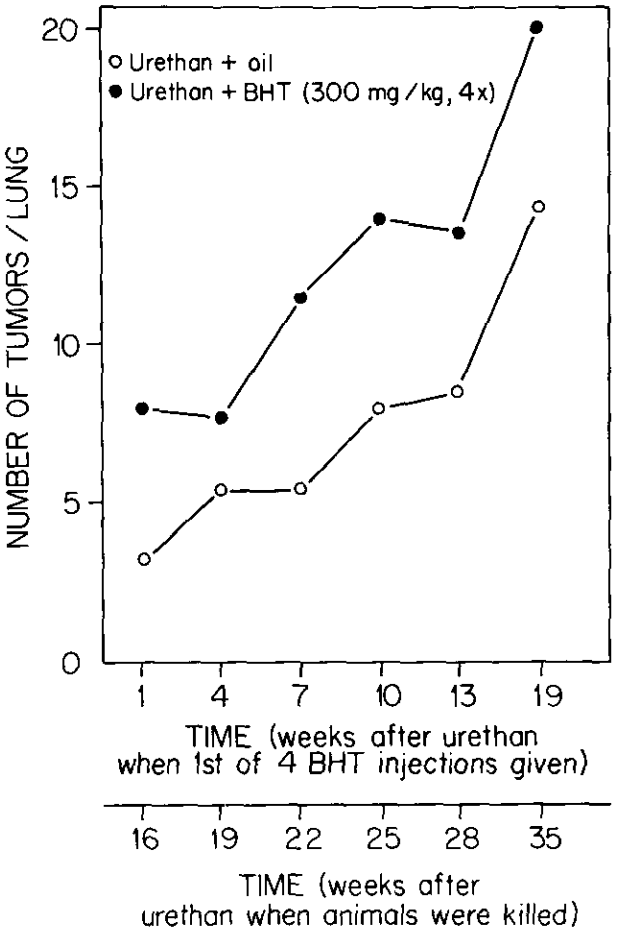

FIGURE 2. Effects of delay of BHT injection following one single dose of urethan. Data from Witschi and Kehrer (10).

weekly injections) were begun 1, 4, 7, 10, 13 or even 19 weeks later. Four months were allowed for tumor expression between the first BHT injection and killing of the animals. Accordingly, animals were killed 16, 19, 22, 25, 28 or 35 weeks after urethan. The data in Figure 2 show that in the BHT-treated animals the number of tumors per lung is invariably higher than in animals treated with corn oil. BHT appears thus to fulfill a second criterion of a true promoting agent.

Dose-Effect Relationships

In several experiments we examined the dose-effect relationship between urethan and BHT. To study the effects of various doses of BHT on lung tumor development, mice treated with 1000 mg/kg of urethan were given six injections of BHT ranging from 50 mg to 300 mg/kg per injection. The lowest dose of BHT, which amounted to a cumulative exposure of 300 mg/kg of BHT was as effective in increasing tumor multiplicity as was the highest dose of BHT, which amounted to a cumulative dose of 1.8 g/kg of BHT (*10*). In a continuing feeding study, the cumulative intake of BHT during an 8-week period following urethan was estimated to be 35 to 40 g/kg. In a second experiment, BHT-containing food was offered only once a week during 24 hr for consecutive 8 weeks, and the cumulative intake of BHT was estimated to be 5 g/kg (*7*). We found that BHT treatment, regardless of total BHT intake, increased tumor multiplicity 50 to 100% compared to controls (Fig. 3). From these data it must be concluded that so far there is no indication of a dose response to BHT. The lowest dose of BHT needed to significantly increase the number of urethan induced lung tumors in mice has yet to be established. There is one important proviso to this conclusion: one or even two single injections of BHT have not been found to enhance tumor multiplicity; the minimum number of BHT injections required to have an effect is four (*9*). More recently we have found that a diet containing 0.75% BHT is effective if given for 2 weeks only after urethan (Witschi, unpublished observations). Further experiments designed to define the minimum time and dose required for BHT intake in food necessary to enhance tumor development are needed and have been initiated.

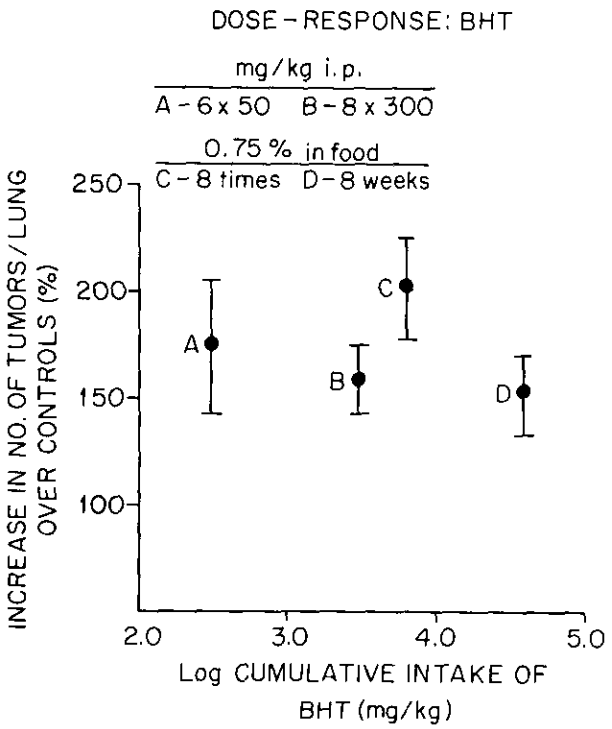

FIGURE 3. Dose-response to BHT: (*A*) 50 mg/kg, IP, six times; (*B*) 300 mg/kg, IP, eight times; (*C*) 0.75% in food, eight times; (*D*) 0.75% in food, 8 weeks. Data from Witschi and Kehrer (*10*) and Witschi (*7*).

Criteria of a Promoting Agent BHT Does Not Meet

While the evidence summarized so far strongly suggests that BHT acts as a tumor promoter in mouse lung, it should not be overlooked that BHT fails to meet one criterion deemed to be important: it does not enhance tumor formation following administration of subcarcinogenic doses of urethan. In a first experiment Swiss Webster mice were treated with 50, 100, 250 or 1000 mg/kg of urethan, given 13 weekly BHT injections and killed 4 months later (*9*). Tumor multiplicity was only significantly increased after 100 mg/kg of urethan or more (Fig. 4). The experiment was more recently repeated using a slightly different protocol (Witschi, unpublished observations). Strain A mice were injected with 5, 25, 50 or 1000 mg/kg of urethan and placed 24 hr later for 8 weeks on a diet containing 0.75% of BHT. The animals were killed 4 months after urethan, and we found that BHT increased tumor multiplicity only in animals treated with the highest dose of the carcinogen (Fig. 4).

BHT is not effective in mouse strains which have a low spontaneous incidence of lung tumors and are resistant to the carcinogenic action of urethan. No increase in tumor multiplicity or tumor incidence was found in BALB/C, C57Bl and C3H mice treated with urethan, even if animals were given repeated injections of 300 mg/kg of BHT (*9*). These observations make one somewhat reluctant to label BHT a typical promoting agent. However, no chemical to be considered a promoter in such organs as liver, stomach, urinary bladder or mammary gland seems

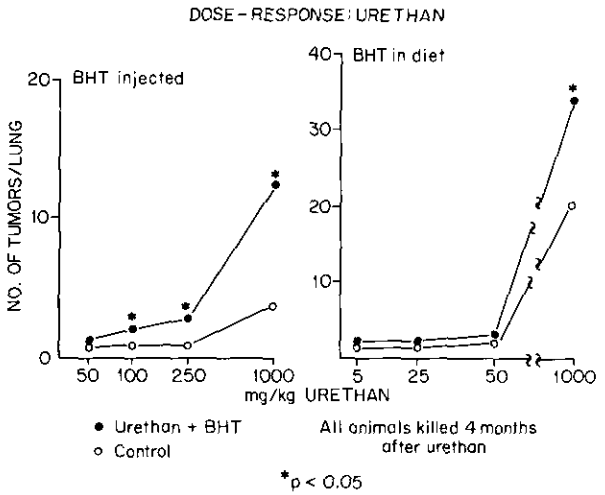

FIGURE 4. Dose-response to urethan: (•) urethan + BHT; (o) control. Animals were injected with various doses of urethan and then given IP injections of BHT (9) (left) or given BHT in the diet (right) (Witschi, unpublished observation). All animals were killed 4 months after urethan. Asterisks (*) denote $p<0.05$.

to fulfill strictly all criteria originally developed for skin tumor-promoting agents (*11*). This may make it necessary to adopt perhaps less rigid criteria as to what constitutes a true two-stage carcinogenesis system. Two-stage carcinogenesis is an important toxicological concept and is an excellent example to document and to further study the role of chemical interactions when exposure to two or more agents is separated in time. To formulate underlying principles of toxicological interactions may be more important than to judge only whether or not a particular experimental system fulfills the rigid criteria developed originally for mouse skin only.

Mechanism of Action of BHT

The mechanisms by which BHT enhances lung tumor formation in mice remain unknown. The original rationale to study the effects of BHT on tumor formation was as follows: in 1972 it was reported for the first time that BHT was capable of producing acute lung damage in mice (*12*). A few days after a single dose of 400 mg/kg of BHT, lung lesions indicative of proliferative changes in the alveolar epithelium were found. Later studies showed that within the first 24 hr after one single injection of BHT there is extensive and diffuse necrosis of type I alveolar epithelial cells. This is followed 2 to 4 days later by proliferation and division of type II alveolar cells. Other pulmonary parenchymal cells such as interstitial cells and capillary endothelial divide 5 to 6 days after BHT injections (*13, 14*).

Morphologic observations together with data on accumulation and disappearance of BHT from the lung (*15*) seemed to make it unlikely that BHT would act directly on type II pneumocytes, the cells believed to be the precursor cells of lung tumors in mice. Rather, it was assumed that repeated proliferation of type II cells, secondary to BHT-induced type I cell necrosis, would be the mechanism of tumor promotion in lung. This assumption was based on the suggestion that in mouse skin all promoting agents are chemicals capable to elicit gene activation and cell proliferation (*16*). It was therefore logical to link BHT-induced type II cell proliferation and enhancement of tumorigenesis. However, some more recent experiments have shown that it is possible to enhance tumor formation in mouse lung by BHT even in the absence of overall cell proliferation. On the other hand, no enhancement of tumor formation can be produced by several agents which do produce cell proliferation in lung. The evidence is as follows. Initially it was assumed that each weekly injection of BHT would be followed by a burst of cell proliferation in mouse lung. Newer data have shown that this assumption is not correct. While substantial cell proliferation is readily demonstrated on days 2 to 5 after one single injection of BHT, subsequent BHT injections given at weekly intervals fail to produce further cell divisions. This has been demonstrated in two independent experiments with the use of autoradiography (Witschi, unpublished observations) or biochemical techniques (*17*) to assess cell proliferation. Since more than one injection of BHT is required to enhance tumor formation, it must be assumed that cell proliferation is not a key element to explain the effects of BHT. Furthermore we have found that no cell proliferation is detectable in mouse lung if animals are treated shortly before or after BHT with SKF 525A (*18*) or are treated with low doses of BHT (50 mg/kg). Nevertheless, if animals are initiated with urethan and then given repeated small doses of BHT or given a combination of BHT and SKF 525A, tumor multiplicity is greater than in controls (*10*). BHT thus has an effect whether it produces cell proliferation in mouse lung or not (*10*).

On the other hand, two agents which are known to produce a burst of cell proliferation in lung will not influence tumor development. If mice are exposed after urethan repeatedly to 100% oxygen, tumor formation is not enhanced (*10*). Repeated intraperitoneal injections of methylcyclopentadienyl maganese tricarbonyl (MMT) are also without effect on tumor development (*5*). There exists a discrepancy between cell proliferation in lung and tumor promotion.

Finally, it is necessary to consider whether the

antioxidant properties of BHT are related to its tumor enhancing effects. At the moment this is not a likely possibility. The structural analog of BHT, butylated hydroxyanisole (BHA) does not promote tumor formation whether it is injected intraperitoneally or administered to urethan treated animals in the diet (*7, 10*). Vitamin E and ethoxyquin are also without any effect (Fig. 5).

The precise mechanism of action of BHT in mouse lung remains to be established. Some *in vitro* data obtained with BHT might provide clues for the design of mechanistic experiments. BHT is cytotoxic and may cause swelling and lysis of cells and depression of metabolic activities such as RNA and protein synthesis (*19*). In phytohemagglutinin-stimulated lymphocytes a mixture of BHT and Tween-80 produces a marked uncoiling of chromosomes as well as extensive cell membrane damage (*20, 21*). BHT perturbs artificial membrane systems (*22*) and inactivates lipid-containing mammalian and bacterial viruses (*23*). There is thus evidence to suggest that BHT interferes with the structural and functional integrity of membrane systems. How exactly these mechanisms relate to enhancement of tumor formation remains to be seen. However, detailed studies at a mechanistic level might not be easily accomplished. The lung is a very heterogenous organ composed of many different cell types and isolation of pure cell populations can present technical difficulties. Moreover, the cells of origin of lung tumors in mice are not known with certainty yet. In Strain A mice, more than 80% of lung tumors seem to develop from type II alveolar epithelial cells. In Swiss Webster mice it appears that most lung tumors develop from the nonciliated cells lining the small airways, the so called Clara cells (Haschek and Witschi, unpublished observations). Since we have found a similar response to BHT in both Strain A and Swiss Webster mice we must assume for the moment that BHT somehow enhances tumor formation regardless of the cells of origin of lung tumors.

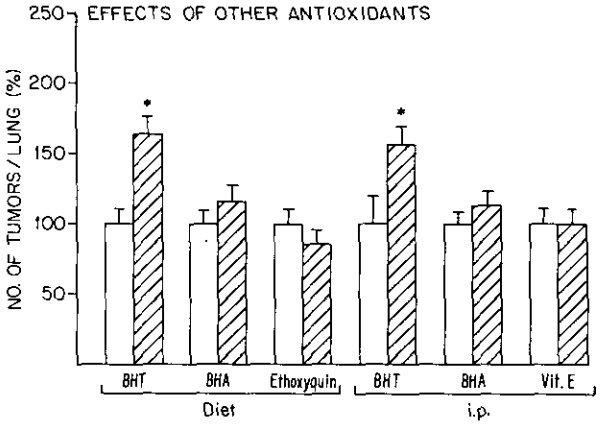

FIGURE 5. Effects of BHT, BHA, ethoxyquin or vitamin E on lung tumor development in lung following urethan. White bars denote controls. Data from Witschi et al. (*5, 7*).

An unresolved problem remains whether BHT increases the actual number of tumors formed or simply accelerates tumor growth. In all experiments tumors were counted on the lung surface. If more tumors are found in animals 4-6 months after BHT, it could be that a surface count was not detecting tumors lying deep in the lung parenchyma. It might not be possible to conclude whether BHT simply accelerated growth of preformed tumors. To answer this question we recently compared in several experiments the number of tumors visible on the lung surface to the number of all tumors visible after clearing the lung. The ratio between the two counts was practically 1:1 (*7*). Surface counting reflects therefore accurately the number of tumors visible to the naked eye or visible under a dissecting microscope. It was concluded that BHT produced within 4 months more visible tumors. On the other hand, we have found that the difference between tumor multiplicity in BHT-treated animals and controls is greater 4 months after urethan than it is 6 or even 9 months after urethan. (Fig. 6). Therefore, we cannot exclude with confidence the possibility that BHT might accelerate, early after urethan, the growth of clusters of transformed cells into visible tumors. This problem remains to be studied further.

In most experiments a dose of urethan was given designed to produce a 90 to 100% tumor incidence in both control and BHT treated animals. This experimental design allows to detect whether BHT treatment increases tumor multiplicity, but does not allow to determine whether BHT affects tumor incidence. In animals treated with other initiators such as 3-methylcholanthrene, dimethylnitrosamine or with lower doses of urethan or with no carcinogen at all, a significantly increased number of tumor-bearing animals was occasionally observed following BHT treatment, but not regularly. In conclusion, while it is certain that BHT increases the number of tumors per lung, it is not yet clear whether this is caused by accelerated tumor growth or by increased formation of tumors.

Effects of Different Initiators

The data base available to document that BHT enhances tumor formation in mice treated with other carcinogens than urethan is limited. A feeding study provided some suggestive but not conclusive evidence that BHT might enhance lung tumor formation in animals pretreated with diethylnitrosamine (*24*). In Swiss Webster mice given dimethyl-

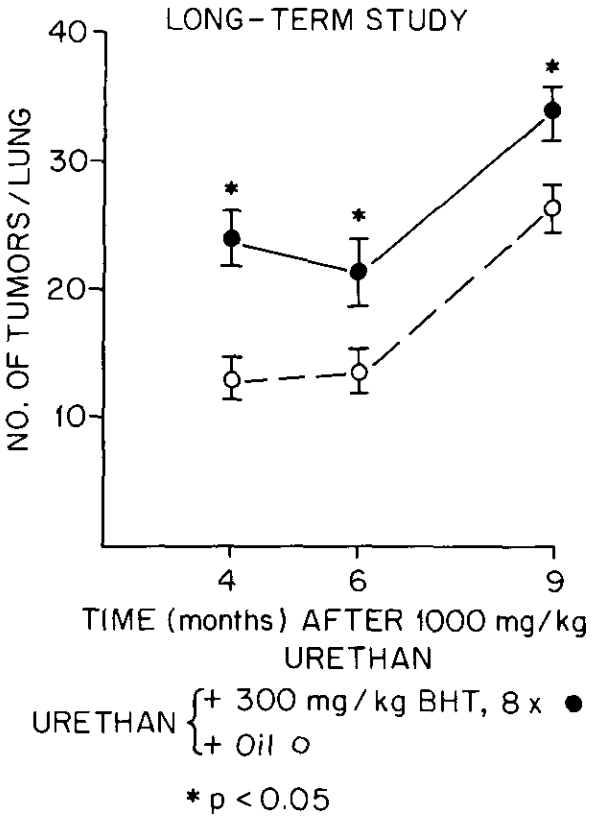

FIGURE 6. Strain A mice were injected with 1000 mg/kg of urethan and given eight weekly IP injections of 300 mg/kg of BHT: (•) urethan × 300 mg/kg BHT, eight times; (o) urethan × oil. The animals were killed 4, 6, or 9 months after urethan. Asterisks (*) denote $p<0.05$ (Witschi, unpublished observations).

nitrosamine or 3-methylcholanthrene, BHT treatment had no effect (*10*). However, in Strain A mice given the same carcinogens, BHT was capable of enhancing tumor formation (*5*). The reasons for these conflicting observations are not clear. In Swiss Webster mice, both methylcholanthrene and dimethylnitrosamine might have produced not enough tumors in order to show the effect of BHT. However the same reasoning cannot be applied to understand the data obtained in Strain A mice: in Strain A mice tumor formation after dimethylnitrosamine was significantly increased even if the dose of dimethylnitrosamine used produced only a limited number of tumors per lung. Further studies are needed to establish unambiguously whether BHT enhances tumor formation in mouse lung following administration of other carcinogens than urethan.

Conclusions

Data reviewed show that BHT fulfills many criteria of a typical promoting agent: it effectively enhances tumor formation in animals exposed to BHT after being injected with urethan but not if they are treated with BHT before urethan. Administration of BHT can be delayed for several months after urethan treatment and still can enhance tumor formation. Route of administration does not play a role and more tumors compared to controls develop whether BHT is given by intraperitoneal injection, oral gavage or whether the animals eat it in the diet. The lowest effective dose of BHT has not yet been determined. On the other hand, it has not been possible yet to show that BHT enhances tumor development in animals exposed to subcarcinogenic doses of an initiating compound. Evidence to show that BHT enhances tumor formation following administration of other carcinogens such as nitrosamines or polycyclic hydrocarbons is positive in one mouse strain and negative in another. Whether BHT accelerates growth of preformed tumors or whether it induces formation of more tumors remains to be reexamined. BHT does not fulfill all the criteria usually set for promoters in the skin tumor model.

However it must be mentioned that BHT acts as a promoting agent in two other systems. In one study, rats were treated with acetylaminofluorene and placed on a diet containing 0.5% BHT. BHT substantially enhanced tumor formation in the liver (*25*).

In an *in vitro* system, BHT inhibits metabolic cooperation between cells, a feature commonly attributed to promoting agents (*26*).

The findings that BHT acts like a promoting agent in three different experimental systems may require a reevaluation of the safety of BHT as a food additive.

In conclusion, there is now good experimental evidence to show that several elements of two-stage carcinogenesis apply to the development of lung tumors in mice. So far, three agents believed to be promoters in other tissues enhance tumor formation in lung: phorbol, a classical skin promoter; saccharin, which is an accepted promoting agent for bladder tumor; and BHT, which has been found in at least one study to enhance tumor formation in liver. The mouse lung tumor system has been suggested as a screening assay for detection of complete carcinogens (*27*). It should be considered whether the use of the mouse lung assay could be expanded by using BHT and possibly other agents as promoters. The assay could then serve to detect complete carcinogens as well as initiators, cocarcinogens, promoters and possibly even copromoters.

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REFERENCES

1. Armuth, V., and Berenblum, I. Systemic promoting action of phorbol in liver and lung carcinogenesis in AKR mice. Cancer Res. 32: 2259-2262 (1972).
2. Witschi, H. P., Williamson, D., and Lock, S. Enhancement of urethan tumorigenesis in mouse lung by butylated hydroxytoluene. J. Natl. Cancer Inst. 58: 301-305 (1977).
3. Hicks, R. M., Chowaniec, J., and Wakefield, J.-St. J. Experimental induction of bladder tumors by a two stage system. In: Carcinogenesis—A Comprehensive Survey, Vol. 2, Mechanisms of Tumor Promotion and Cocarcinogenesis (T. J. Slaga, A. Sivak, and R. K. Boutwell, Eds.), Raven Press, New York, 1978, pp. 475-489.
4. Theiss, J. C., Arnold, L. J., and Shimkin, M. B. Effect of commercial saccharin preparations on urethan-induced lung tumorigenesis in strain A mice. Cancer Res. 40: 4322-4324 (1980).
5. Witschi, H. P., Hakkinen, P. J., and Kehrer, J. P. Modification of lung tumor development in A/J mice. Toxicology 21: 37-45 (1981).
6. Witschi, H. P., and Lock, S. Toxicity of butylated hydroxytoluene in mouse following oral administration. Toxicology 9: 137-146 (1978).
7. Witschi, H. P. Enhancement of tumor formation in mouse lung by dietary butylated hydroxytoluene. Toxicology 21: 95-104 (1981).
8. Stoner, G. D., Shimkin, M. B., Kniazeff, A. J., Weisburger, J. H., and Weisburger, E. K. Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Res. 33: 3069-3085 (1973).
9. Witschi, H. P., and Lock, S. Enhancement of adenoma formation in mouse lung by butylated hydroxytoluene. Toxicol. Appl. Pharmacol. 50: 391-400 (1979).
10. Witschi, H. P., and Kehrer, J. P. Adenoma development in mouse lung following treatment with possible promoting agents. J. Am. College Toxicol. I: 171-184 (1982).
11. Slaga, T. J., Sivak, A., and Boutwell, R. K. (Eds.) Carcinogenesis—A Comprehensive Survey, Vol. 2, Mechanisms of Tumor Promotion and Cocarcinogenesis. Raven Press, New York, 1978.
12. Marino, A. A., and Mitchell, J. T. Lung damage in mice following intraperitoneal injection of butylated hydroxytoluene. Proc. Soc. Exptl. Biol. Med. 140: 122-125 (1972).
13. Adamson, I. Y. R., Bowden, D. H., Cote, M. G., and Witschi, H. P. Lung injury induced by butylated hydroxytoluene. Cytodynamic and biochemical studies in mice. Lab. Invest. 36: 26-32 (1977).
14. Hirai, K. I., Witschi, H. P., and Cote, M. C. Electron microscopy of butylated hydroxytoluene-induced lung damage in mice. Exptl. Mol. Pathol., 27: 295-308 (1977).
15. Williamson, D., Esterez, P., and Witschi, H. P. Studies on the pathogenesis of butylated hydroxytoluene-induced lung damage in mice. Toxicol. Appl. Pharmacol. 43: 577-587 (1978).
16. Boutwell, R. K. The function and mechanism of promoters of carcinogenesis, CRC Crit. Rev. Toxicol. 2: 419-433 (1974).
17. Gerhardt, W. T. Low dose chemical and radiation carcinogenesis in the murine lung. MSc Thesis, University of Tennessee, Knoxville, TN, 1981.
18. Kehrer, J. P., and Witschi, H. P. Effects of drug metabolism inhibitors on butylated hydroxytoluene-induced pulmonary toxicity in mice. Toxicol. Appl. Pharmacol. 53: 333-342 (1980).
19. Milner, S. M. Effects of the food additive butylated hydroxytoluene on monolayer cultures of primate cells. Nature 216: 557-576 (1967).
20. Sciorra, L. J., Kaufmann, B. N., and Maier, R. The effects of butylated hydroxytoluene on the cell cycle and chromosome morphology of phytohaemagglutinin-stimulated leucocyte cultures. Food Cosmet. Toxicol. 12: 33-44 (1974).
21. Sciorra, L. J., Maier, R., Thompson, S., and Kaufman, B. H. An analysis of the uncoiling effect of a BHT-Tween 80/absolute ethanol mixture on Velban arrested human metaphase cells. Cytologia, 41: 63-66 (1976).
22. Eletr, S., Williams, M. A., Watkins, T., and Keith, A. D. Perturbations of the dynamics of lipid alkyl chains in membrane systems: effect of the activity of membrane-bound enzymes. Biochim. Biophys Acta 339: 190-201 (1974).
23. Snipes, W., Person, S., Keith A., and Cupp, J. Butylated hydroxytoluene inactivates lipid-containing viruses. Science 188: 64-65 (1975).
24. Clapp, N. K., Tyndall R. L., Cumming, R. B., and Otten, J. A. Effects of butylated hydroxytoluene alone or with diethylnitrosamine in mice. Food Cosmet. Toxicol. 12: 367-371 (1974).
25. Peraino, C., Fry, R. J., Staffeldt, E., and Christopher, P. Enhancing effects of phenobarbital and butylated hydroxytoluene on 2-acetylaminofluorene-induced hepatic tumorigenesis in the rat. Food Cosmet. Toxicol. 15: 93-96 (1977).
26. Trosko, J. E., Yotti, L. P., Warren, S. T., Tushimoto, G., and Chang, C. Inhibition of cell-cell communication by tumor promoters. In: Carcinogenesis—A Comprehensive Survey, Vol. 7, Cocarcinogenesis and Biological Effects of Tumor Promoters (E. Hecker, N. E. Fusenig, W. Kunz, F. Marks and H. W. Thielmann, Eds.), Raven Press, New York, 1982, pp. 565-585.
27. Shimkin, M. B., and Stoner, G. D. Lung tumors in mice: application to carcinogenesis bioassay. Adv. Cancer Res. 21: 1-58 (1975).